CYTOGENETIC STUDIES OF MAIZE AND NEUROSPORA

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INDUCTION OF MUTATIONS IN THE SHORT ARM OF CHROMOSOME 9 IN MAIZE

In the past, many methods have been used to induce mutations. The majority of these methods do not give rise to specific mutations or to mutations confined to specific regions of the chromosome complement. Instead, a random assortment and distribution of mutations are obtained. A better understanding of the factors involved in the mutation processes would be possible if specific mutations associated with specific regions of the chromosomal complement could be effected. Recent investigations with maize have suggested several approaches to the problem of induction of specific mutations. One of these will be considered in this report. In previous reports, the repeated induction of the mutants *pyd* (pale-yellow seedling), *wd* (white seedling), and *yg* (yellow-green seedling and plant) has been described. Their origin has been associated with the behavior in several successive nuclear divisions of a recently broken end of a chromosome. This behavior has been called the chromatid type of breakage-fusion-bridge cycle. The *pyd* mutant appeared when the chromosomal complement was deficient for a small terminal segment of the short arm of chromosome 9; the *wd* mutant appeared when a slightly longer terminal segment was missing. The mutant phenotype *bz* (bronze) has likewise appeared following the production of a specific internal deficiency, as previously described. From this and other types of evidence, it has been concluded that specific mutations will arise as the consequence of specific minute deficiencies. If the breakage-fusion-bridge cycle could give rise to a number of different internal minute deficiencies, and if the short arm of chromosome 9 were subjected to this process, various new mutants other than *pyd, wd, yg,* and *bz* should appear, each related to loss of a specific minute segment within this arm. The methods used to isolate the mutants *pyd, wd, yg,* and *bz* were selective. Therefore, a random sample of mutants which might be produced as the consequence of the breakage-fusion-bridge cycle did not appear. During the past year, nonselective methods have been used to determine whether the expected new mutants actually are being produced.

Cytological observations of the breakage-fusion-bridge cycle, as well as theoretical considerations, have indicated that this cycle will result in the production of internal deficiencies. Occasionally, a chromatid bridge in an anaphase figure is broken at more than one place. If a chromatid bridge breaks in three places, two centric chromosomes with a single broken end and two acentric fragments, each with both ends broken, will be formed. It is possible for the two fragments to enter one telophase nucleus along with the centric chromosome. If, in this nucleus, a particular type of fusion of broken ends occurs, a centric rod chromosome with an internal deficiency and an acentric ring fragment can be produced (following fusion of the two broken ends of the proximal fragment to form an acentric ring, and fusion of one broken end of the distal fragment with the broken

end of the centric chromosome). If the remaining free broken end of the centric rod chromosome healed and no longer underwent the breakage-fusion-bridge cycle, a chromosome with an internal deficiency might subsequently be isolated. Sufficient cytological evidence has accumulated to support the assumption that this is one method of origin of internal deficiencies. Theoretical considerations suggest a second method for obtaining internal deficiencies. Many investigators have considered the anaphase chromosomes to be multiple, that is, composed of two or more sister strands. It is probable that effective doubleness at anaphase is present in some cells or tissues and not in others. Should a chromatid bridge at anaphase be composed of two sister strands, breakage need not occur at comparable positions in the two strands. Should the breakage be unequal, the chromatin composition of the two sister strands entering a nucleus would not be comparable. They could differ by various duplications or deficiencies. If, in the following telophase, fusion occurred between the two broken ends of the unequal strands, the chromatin components between the two centromeres would consist of two dissimilar instead of similar segments. A chromatid bridge and breakage of this bridge would follow in the next mitotic division. Should the resulting newly broken end heal permanently, it might be possible subsequently to isolate a chromatid with an internal deficiency. The type and extent of deficiency would depend on the positions of breakage in these two divisions. This process would give rise to internal deficiencies without fragment formation. Again, theoretical considerations have suggested that the *chromosome* type of breakage-fusion-bridge cycle (see previous reports) should result in chromosomes with internal deficiencies ranging from minute to extensive. Therefore, both the chromatid and the chromosome type of breakage cycle have been utilized in an attempt to produce and isolate new mutations confined within the short arm of chromosome 9.

To isolate new mutants produced by the *chromatid* bridge cycle, F_2 progeny derived from F_1 plants that had received a recently broken chromosome 9 from one parent were examined. To isolate new mutants produced by the *chromosome* bridge cycle, the selfed progeny of individuals that had received a newly broken chromosome 9 from each parent were examined. In many cases, the constitution of the short arm of the chromosomes 9 with healed broken ends had been considerably altered during the period of the breakage cycles. Large as well as small duplications or deficiencies frequently were present. Many of these altered chromosomes 9 did not pass through the gametes to the next generation. Whenever the pollen grains and eggs carrying the chromosomes 9 with altered short arms were capable of effecting fertilization, the selfed progeny could include individuals homozygous for these altered short arms. Should an alteration, when homozygous, result in a changed phenotype, individuals with a distinct mutant character would appear in the progeny. Considerations of space and labor confined the search for new mutations mainly to the kernels and the seedlings. A number of new mutants appeared in these progenies. The most clearly defined of these mutants were selected to determine whether or not they were located in the short arm of chromosome 9. Only 3 of the distinctly new types of mutant have been sufficiently analyzed to indicate their positions in the short arm. These are a small-kernel mutant (*smk*), a spotted-leaf mutant (*spl*), and a pale-green mutant (*pg*). The *smk* and *spl* mutants

are located in the distal third of the short arm, whereas *pg* is located between the mutants *sh* and *wx*. Many new *pyd* and *wd* mutants and a few new *yg* mutants appeared in these cultures. Although 69 mutants arising from newly broken chromosomes 9 have been tested, they represent only 7 distinct phenotypes because of the repeated occurrence of the same mutations. In the published linkage group of chromosome 9, 7 spontaneously arising mutants have been placed in the short arm. The symbols for these are: *Dt, yg, C, sh, bz, bp,* and *wx*. The newly broken chromosomes 9 have given the 7 mutants *pyd, wd, yg, smk, spl, bz,* and *pg*. As has been stated previously, the *yg* and *bz* mutants derived from the broken chromosomes 9 are allelic to the 2 mutants, *yg* and *bz,* that arose spontaneously in genetic cultures.

An interesting type of chromosomal behavior has appeared in three of the broken-chromosome cultures mentioned above. In each culture, one of the broken chromosomes 9 is continually being lost from cells during development. This loss is not due to bridge formation or to ring chromosome behavior, but appears to be caused by the inability of the two halves of this chromosome to migrate to opposite poles in some of the somatic anaphase figures. The rate of loss varies widely from plant to plant. Within a single plant, changes in rate occur; this is made evident by the presence of distinct sectors each with its own rate of loss. To date, only a cursory examination of the nature of this phenomenon has been made; it warrants further study. In addition, some of the mutants appearing in these cultures are individually provocative. Several show variegation characterized by a change from mutant to normal-appearing tissues. For any one plant, a distinctive or basic rate of change is apparent, but this basic rate differs from plant to plant. Sectors with changed rates of variegation appear in all plants, especially in the later-appearing tissues. It is significant that twin sectors accompany many if not most of the alterations in rate; this is expressed by the appearance of a sector of tissue having a greatly increased rate of variegation immediately adjacent to a sector of tissue having a much reduced rate of variegation.

Preliminary Studies of the Chromosomes of the Fungus Neurospora crassa

During the fall of 1944, a period of ten weeks was spent in the Biological Laboratories of Stanford University, where genetic studies are being conducted with the fungus *Neurospora*. The purpose of this visit was to obtain some knowledge of chromosomal and nuclear behavior in *Neurospora crassa*. Although fungi have assumed an important role as genetic materials, little has been done to coordinate the genetic studies with a study of chromosomal conditions. As genetic investigations with fungi progress, the necessity for correlative cytogenetic analyses will become increasingly evident. It was a pleasure to have the opportunity of examining *Neurospora* in this laboratory. Progress was greatly accelerated by the availability of large numbers of stocks, both wild-type and mutant, and by the generous and cooperative support of the members of the department.

The observations were confined to the chromosomes and nuclei of the ascus. They included observations of chromosome numbers, absolute and relative sizes of the chromosomes, centromere positions, internal organization of the chromosomes, zygote formation, chromosome behavior in the two meiotic mitoses and the equational mitosis which follows, and scattered observations of several chromosomal translocations. In the short time available, no

one of these topics could be adequately considered. Nevertheless, this over-all survey has suggested that some fungi may be adequate and, in several respects, superior material for cytogenetic studies.

The haploid number of chromosomes in *Neurospora crassa* is 7. Each chromosome of the complement is distinguished by its relative length, the position of its centromere, and its internal organization. The longest chromosome is approximately 2.7 times as long as the shortest. The second-longest chromosome, chromosome 2, has a nucleolus organizer located close to the end of the short arm. The organizer region functions to produce a nucleolus in a manner similar to that observed in many other organisms. Because of its location close to the end of one arm of this chromosome, a minute satellite is formed. Throughout the various nuclear cycles, the relative lengths of the chromosomes of the complement are maintained. Therefore, absolute lengths need be given only for the longest chromosome. In the third division in the ascus, which is equational, this chromosome may be only 1.5 microns long. At the full meiotic prophase extension, it may be 15 microns long. Chromomere patterns were observed at this latter stage; each chromosome appears to have its characteristic pattern. Centromere positions were adequately determined for the two longest chromosomes, and approximate positions were obtained for the other five chromosomes. Two heterochromatic segments were observed and located adjacent to the centromere, but the chromosome or chromosomes carrying these heterochromatic segments were not identified.

Fusion of two haploid nuclei to form the zygote nucleus occurs in the very young ascus. The two sets of chromosomes in this zygote nucleus then commence the activities associated with meiosis. The behavior of the chromosomes in the early meiotic stages is of considerable theoretical interest. During meiosis in most organisms, homologous associations commence when the chromosomes are in a very elongated state. In the *Neurospora* strains most intensively studied, this occurs when the chromosomes are greatly contracted. Following nuclear fusion, the chromosomes contributed by each nucleus undergo what appears to be a typical prophase contraction without visible evidence of splitting, until, in some strains, the chromosomes are almost as short as those of the metaphase of the third division in the ascus. In this highly contracted state, the homologous chromosomes commence their synaptic associations. Before the chromosomes have reached this state, fusion of the nucleoli contributed by the two nuclei usually has occurred. Actual physical association of the homologues usually begins at one or both ends and continues along the chromosomes. In many nuclei, synapsis is completed for some pairs of chromosomes before the members of the other pairs have approached sufficiently close to each other to commence actual contacts. It is not clear from these studies whether the approach of homologous chromosomes toward each other is directed or whether it follows from random movements of the chromosomes in the nucleus. It is of considerable theoretical interest to determine the range of the synaptic force which brings about homologous associations of chromosomes. It is suspected that the young asci of *Neurospora* might be readily cultured. Because of the relatively large volume of the nucleus and the small size of the chromosomes in these asci, continuous observations of the behavior of these chromosomes in the living nuclei might be possible.

Following the synaptic phase, the associated homologous chromosomes begin to elongate until, as stated above, the longest

chromosome may reach a length of 15 microns. Diplotene sets in rather suddenly following the completion of elongation of the synapsed chromosomes. The period from diplotene to metaphase I is passed through very rapidly. At diakinesis, typical chiasmata may be observed leading to rather orthodox, even though small, metaphase I bivalents. Although the nucleolus becomes smaller during the prometaphase stage, it is still present at metaphase. Chromosome 2 remains attached to the nucleolus by its organizer region. Anaphase I appears to be essentially typical except for the presence of the nucleolus. The nucleolus may be dragged toward one pole or stretched between the poles because the nucleolus organizer of one or more chromatids of chromosome 2 still remains attached to it. The nucleolus becomes detached before telophase sets in. At telophase I, and likewise at telophases II and III, the centromere regions of all the chromosomes form an aggregate that lies at the apex of a distinct protrusion of the nucleus (the beak). No true resting nucleus is formed. Instead, the chromosomes uncoil, the individual arms of each chromosome extending into an elongated nucleus. A new nucleolus is formed and remains attached to the nucleolus organizers of chromosome 2. Contraction of the chromosomes initiates prophase II. This continues until the two dyad chromosomes are in the form of short, parallel rods, each showing a conspicuous centromere region. Metaphase and anaphase II are essentially typical. At telophase II the centromere regions are again aggregated at the apex of the beak of the nucleus; the chromosomes uncoil and the two arms of each chromosome extend into the nucleus as individual strands. They remain in this condition until the following prophase. The extent of elongation of the chromosomes appears to be similar to that observed in the meiotic prophase. In each nucleus, a new nucleolus is formed at the position of the nucleolus organizers of chromosome 2. Prophase III is initiated by contraction of the arms of the chromosomes. The metaphase and anaphase of division III proceed as a typical equational mitosis. The resting stage of nuclear organization follows telophase III. Shortly after spore delimitation, a mitosis occurs in each ascus. This is also a typical equational mitosis. In essential details, divisions I and II are typically meiotic. Division III is essentially a somatic mitosis, except that the chromosomes retain their identity as elongated strands from the telophase of division II to the prophase of division III. The time of effective splitting of the chromosomes for this division is of some theoretical interest.

Because many of the mutations in *Neurospora* have appeared following X-ray and ultraviolet irradiation, it was suspected that various types of chromosomal translocation might likewise have been induced by these treatments. Three irradiation-induced mutants, whose genetic behavior suggested the presence of some chromosomal abnormality, were selected for examination. A translocation between two nonhomologous chromosomes was found in each case. Intensive studies of these translocations were not undertaken, but the preliminary observations have suggested the usefulness of some translocations for attacking special problems.